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Comparative botanical studies of some *Salvia* species (Lamiaceae) grown in Egypt. II. Anatomical and molecular characteristics.

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ABSTRACT

This paper is the second part in a study concerning with various botanical characters of four plant species of genus *Salvia* L. namely; *Salvia coccinea* Buc'hoz ex Etl., *Salvia farinacea* Benth., *Salvia officinalis* L. and *Salvia splendens* Sellow ex Roem. &Schult. This work comprised a detailed botanical study of the anatomical structure of various plant organs and a classification of studied plant species based on botanical characters and protein electrophoresis through SDS-PAGE as a molecular approach. Anatomical study and analysis by light microscope included: apical and median portions of the main stem, leaf blade, petiole, flower bud and nutlet. Moreover, SEM was used to examine the ultrastructure of stomata, trichomes and nutlet surface. Molecular study using electrophoretic separation of seed storage proteins was carried out to identify and differentiate among the investigated species. The results obtained from SDS-PAGE analysis proved that both *S.coccinea* and *S.farinacea* are highly similar (83%) compared to other studied species. Followed that, the relationship between *S.splendens* and each of *S.coccinea* (71%) or *S.farinacea* (57%). The similarity between *S.officinalis* and any of the other three species was lesser.

Keywords: *Salvia*, anatomical structure, SEM, molecular and SDS-PAGE.

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INTRODUCTION

This is the second paper in a study dealing with various botanical attributes of four species of *Salvia*. The first part of this investigation (El-Sahhar *et al.,* 2016) introduced a detailed information about germination of seeds and morphology of vegetative and reproductive growth of these plants. In this work, anatomical and molecular studies were carried out.

Many species of Lamiaceae are used as medicinal plants, culinary herbs (*e.g., Mentha*, mint; *Ocimum*, basil; *Rosmarinus*, rosemary; *Salvia*, sage; and *Thymus*, thyme), fragrance plants *e.g.*, *Lavandula*, lavender and *Pogostemon*, patchouli and food (*e.g., Stachys affinis*) (Metcalfe and chalk,1957; Dahiya, 1979; Harley *et al.*, 2004 and Simpson, 2010).

Metcalfe and Chalk (1957) mentiond that the branches of Lamiaceae are very frequently rectangular in transverse section, and there is usually a considerable development of collenchyma in the angles, and in some species, elsewhere in the primary cortex. The arrangement of the collenchyma in the stem is of diagnostic value. In the young stem, xylem and phloem are, in some species, confined to collateral bundles, which are especially well developed in the angles of the axis. In other species contiguous bundles are separated by interfascicular fibers, whilst in a third category, the continuous xylem is traversed by narrow medullary rays. The vessels are usually small in diameter. And added that many species are densely covered with hairs of various kinds, one of the most characteristic being the short stalked glands with heads of 1-16 or more cells. These secrete pleasantly scented oils, such as those of lavender, rosemary, thyme and peppermint. Nonglandular hairs are also frequent and may be uniserate, tufted, or branched. Moreover, Salimpour *et al.* (2012) studied the stem anatomy of eight *Salvia* L. species and found that the cross section of stem in all species is regular or irregular quadrangular. Epidermis is single layer with ellipsoid, oblong, ovoid or circular cells. Collenchyma is made of three to ten layered under epidermis, projecting at the corners of stem, becoming thin towards edges. Cortex parenchyma under epidermis is three to six layered with oblong, hexagonal or ellipsoid cells. Thick sclerenchyma groups with three to six layers are presented above vascular bundles at the corners. Phloem is located under sclerenchyma. Vascular bundles at the corners are large and sometimes with lobed and at the edge are very small. Pith rays are seven to nineteen rowed. Two hair types of stem are glandular or eglandular.

Cronquist (1981) indicated that the leaves of Lamiaceae are opposite or sometimes whorled (alternate in *Icomum*), simple or occasionally pinnately compound. Stomata commonly diacytic, less often some or all of them anomocytic. Petiole commonly with a more or less arcuate vascular strand or with a ring of vascular bundles.

Özdemir and Altan (2005) pointed out that number of vascular bundles in the petiole may serve as taxonomically diagnostic characters and observed in *Salvia huberi* a single large vascular bundle in the center of the petiole and 5 small lateral bundles, 2 of which are located in one petiolar wing and 3 in the other.

Marin *et al.* (1996) studied the nutlet surface characters for 13 species of *Salvia* and found that nutlet surface of *Salvia coccinea* consists of hexangular or pentangular cells (papillae) with clear furrow between them. Nutlet of *Salvia officinalis* consistes of isodiametric hexangular or pentangular cells but the center of which is sunken.

In concern of genetic characters of *Salvia* species, Alberto *et al.* (2003) mentioned that chromosome numbers of *Salvia* species are unusual in their extreme variability. Published counts range from a low of 2n = 12 in *Salvia hispanica* to a high of 2n = 88 in the octoploid *Salvia guaranitica*. In addition to wide variation in ploidy level in *Salvia*, the basic number of chromosomes is also wide-ranging with x reported as 6, 7, 8, 9, 10, 11, and 15 for species within the genus.

Masoud *et al.* (2010) stated that various cytological studies performed on the genus *Salvia* indicate surprisingly diverse chromosome numbers in the genus. The genus seems to be polybasic, with different groups of species in different parts having polyploid origins. The Mediterranean group seems to be characterized by x = 7, those in Europe and Russia by x = 11 and those studied in California by x = 16. Some studies show that *Salvia* subgenus *Calosphace* is characterized by x = 11 and lower numbers.

This study aimed at comparing the difference in anatomical characters using light and electronic microscope, in addition to molecular biological evidence of four species of genus *Salvia* L. (Lamiaceae) in continuoity of the first part of this study (El-Sahhar *et al.,* 2016)
since these species are important ornamental and medicinal plants in Egypt.

MATERIALS AND METHODS

Field work

The field work procedure was described in the first part of this study (El-Sahhar *et al.,* 2016).

Anatomical studies

Light microscope studies:

Samples were taken periodically throughout the first growing season. Specimens represented different plant organs, including; the main stem through its apical and median portions, the leaves developed on the main stem and on lateral branches represented by the median portion of the lamina and petiole, flower buds and nutlets.

Microtechnique procedures given by Nassar and El-Sahhar (1998) were followed. Specimens were killed and fixed for at least 48 hrs in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, 85 ml ethyl alcohol 70%). After fixation, materials were washed in 50% ethyl alcohol, dehydrated through a normal butyl alcohol series and embedded in paraffin wax (melting point 56-58^{o}C). Twenty microns tissue sections were cut with a rotary microtome and placed on slides containing Haupt's adhesive and formalin. Sections were stained with crystal violet-erythrosin before mounting in Canada balsam and cover slips attached. Slides were analyzed microscopically and photomicrographed.

Scanning electron microscope (SEM) studies:

Stomata, trichomes and nutlets (seeds) of the four studied *Salvia* plant species were subjected to investigation using scanning electron microscopy (SEM) as a modern botanical discipline. This study adds taxonomic information aid in establishment of taxonomic relations among studied plant species.

The specimens were mounted after dehydration on the copper specimen holder stub with double-sides adhesive discs and coated with a thin layer of gold palladium using Edwards sputter coater unit S 150 A, England. The specimens were examined in different positions using different magnifications to elucidate characters of various types of stomata and trichomes on both leaf epidermal surfaces of studied plants and the nutlet surface by JXA-840 A model Electron Probe Microanalyzer – JEOL, Japan.

Scanning electron microscopy was carried out at the Central Laboratory, National Research Center (NRC), Dokki, Giza, Egypt.

Molecular study

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS - PAGE) of seed storage protein.

SDS-PAGE was used to identify the four studied plant species of *Salvia* through their protein fingerprints. Protein fractions were performed exclusively on 0.75 mm-thick vertical slab gels, being cast and electrophoresed using the BioRad miniprotein II system as given by Laemmli (1970) and modified by Studier (1973). Protein electrophoresis was carried out in Faculty of Agriculture Research Park (FARP), Cairo University, Giza, Egypt.

The resulting data were expressed in the proper units (Kilo- Dalton), where the role of Gel analyzer softwar was considered the precise determination of each band then counting and classifying the bands according to standard molecular weight. The similarity coefficients were used to construct a dendrogram

through the clustering method of UPGMA (Unweighed Pair-Group Method with Arithmetical Averages) using SY stat version 7 (1997), SPSS Inc.

RESULTS AND DISCUSSION

Anatomical information

Structure of the main stem

The apical internode

The apical portion of the main stem, represented by the internode directly below the shoot apex, was studied from the anatomical point of view at the age of five months as it represents the primary structure of the main stem of studied *Salvia* species. The transverse sections shown in Figure (1) reveal that the stem surface of *S. coccinea* and *S. farinacea* is strongly ridged and fluted, it has four ridges alternating with four furrows. The stem of *S.officinalis*, however is tetragonal in outline but in case of *S. splendens*, the stem is hexagonal having six ridges and six furrows.

The epidermis consists of a uniseriate layer of nearly square shaped cells and covered with a thin layer of cuticle. Trichomes of various kinds ;*i.e.*, non-glandular and glandular are present in the epidermis (Figures, 2 to 5). The non glandular trichomes are uniseriate, pointed or conical in shape, straight or curved, sometimes hook-like consisting of 1-5 cells and most of them with compound foot. The glandular trichomes consist of two types; capitate and peltate. The capitate trichomes composed of base of one or two cells, stalk of 1-3 cells and cutinized uni or bicellular secretive head. The peltate trichomes consist of a basal epidermal cell, one stalk cell and a broad head of 4 or more cells. Underneath the epidermis, there are 5-6 layers of collenchyma located opposite to the ridges and there are 3-4 layers of chlorenchyma cells between them. The rest of the cortex is 3-6 layers of parenchymatous cells. The starch sheath, the innermost layer of the cortex, is difficult to recognize in the studied species of *Salvia* except of *S. coccinea*.

The stele is composed of collateral vascular bundles arranged more or less in a square shape. There are four arcs of major collateral bundles located opposite the corners in *S. coccinea, S. farinacea* and *S.officinalis* while in *S.splendens*, there are six vascular bundles opposite to the ridges. In addition to one to three minor bundles between any of two arcs lying opposite to the furrows. The major bundle has 12-20 parallel rows of vessels, while the minor bundle has 5 to 7 parallel rows of vessels. The cambium zone is easily recognized as a continuous ring between phloem and xylem.

The pith occupies large portion in the center of the section and consists of relatively large polygonal parenchymatous cells with relatively small triangular intercellular spaces. Worthy to note that the area of the pith constitutes about 70% of the whole area of the transverse sections.

The median internode

The transverse sections through the median portion of the main stem of investigated *Salvia* species at the age of five months were examined microscopically and photomicrographed (Figure 6).

It is obvious that the structure of the median internode of *S.coccinea, S.farinacea* and *S.splendens* is generally indifferent with that of its apical portion. However, the diameter of the median internode is large than that of the apical one. In case of *S.officinalis,* the secondry growth was observed at early stage compared to other species. Moreover, the stem as appeared in the transverse section looses its tetragonal shape showing a cylindrical outline.

The epidermal cells respond to the increase in stem diameter stresses by tangential enlargement and radial divisions. The epidermis consists of a single layer of nearly square parenchymatous cells covered with a thin layer of cuticle. Trichomes are present. The cortex of *S.officinalis* consists of about 8-10 layers of cells. The outer 4-5 layers are collenchymatous or chlorenchymatous cells and the remainder is parenchymatous cells. As to the other three species, the cortex underlying the angles composed of 8-10 layers of which the outer 5-8 layers are collenchyma cells abutting the epidermis and the rest of layers of cortex are parenchyma cells. The

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cortex in the furrows between the ridges consists of 3-4 layers of chlorenchymatous cells. The starch sheath is easily recognized in *S. coccinea.*

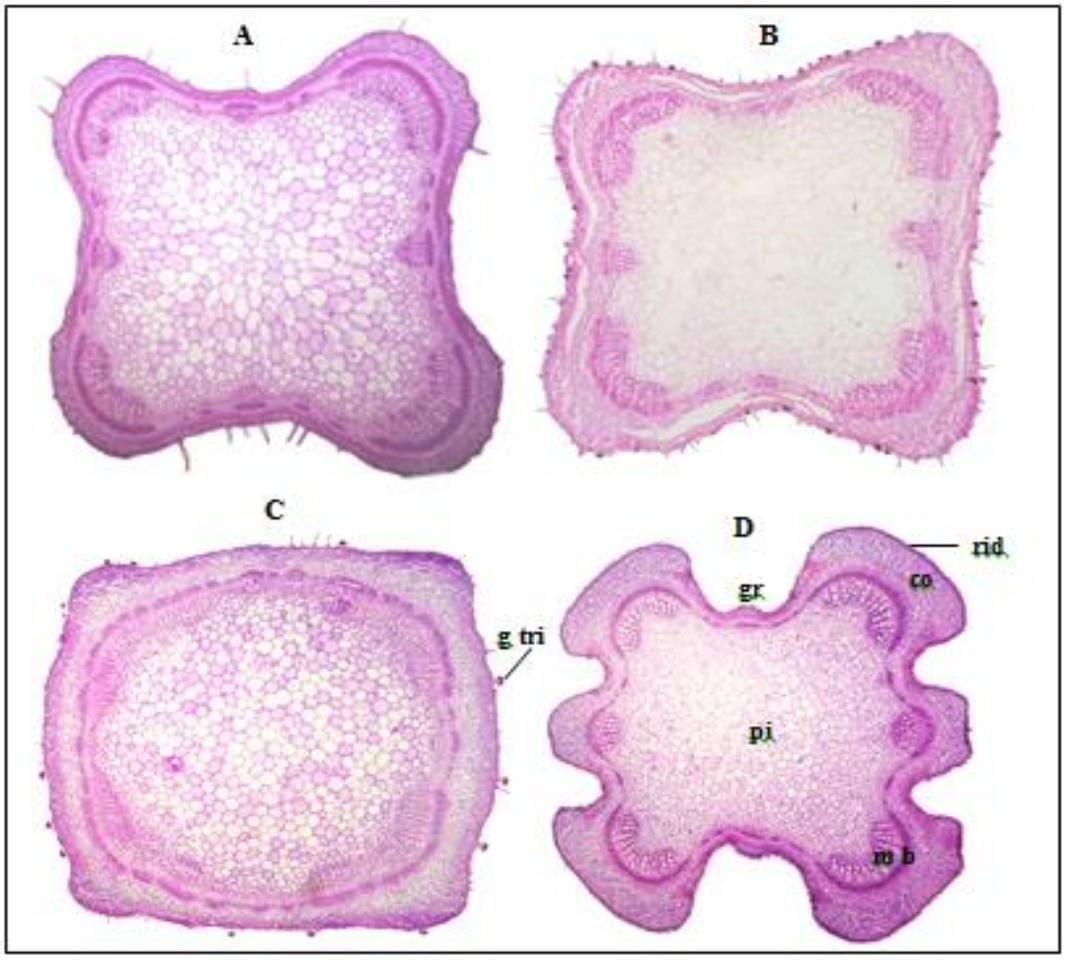

Fig.1. Transverse sections through the apical internode of the main stem of the four studied plant species of *Salvia* at the age of five months (X 35).
A. *S. coccinea* B. *S. farinacea* C. *S.officinalis* D. *S.splendens*
Details: co, cortex; g tri, glandular trichome; gr, groove; m b, main bundle; pi, pith and rid, ridge.

Fig. 2. Types of trichomes accompanied the apical internode of the main stem of *Salvia coccinea.*
A and B: Non-glandular uniserate trichomes of one, three or five cells. (X 100)
C and D: Glandular capitate trichomes composed of a head of one cell with long or short stalk. (X 400)

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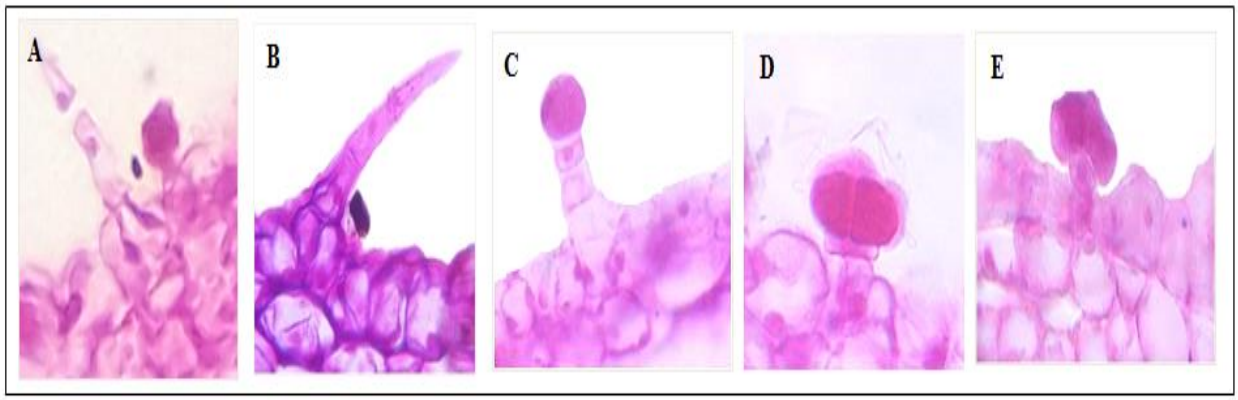

Fig. 3. Types of trichomes accompanied apical internode of of the main stem of *Salvia farinacea.*
A and B: Non-glandular uniserate trichomes of two or three cells. (X 400)
C and D: Glandular capitate trichomes with a head of one or two cells. (X 640)
E: Glandular peltate trichome with a head of four cells. (X 640)

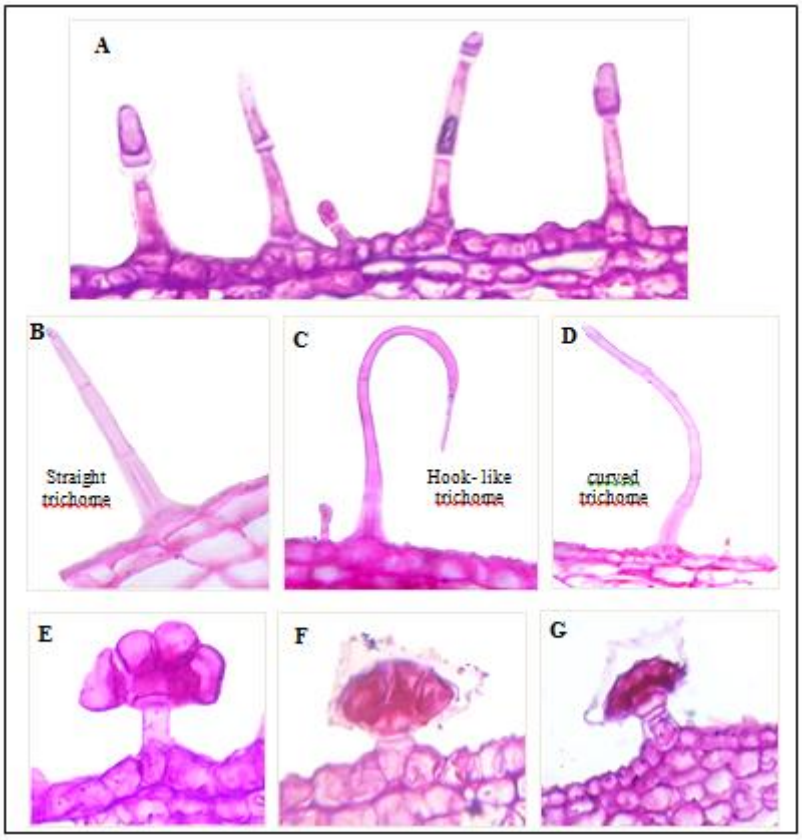

Fig. 4. Types of trichomes accompanied apical internode of the main stem of *Salvia officinalis*.
A, B, C and D: Non-glandular uniserate trichomes of two to five cells. (X 160)
E, F and G: Glandular peltate trichomes with a head of four or more cells. (X 640)

ISSN: 0975-8585

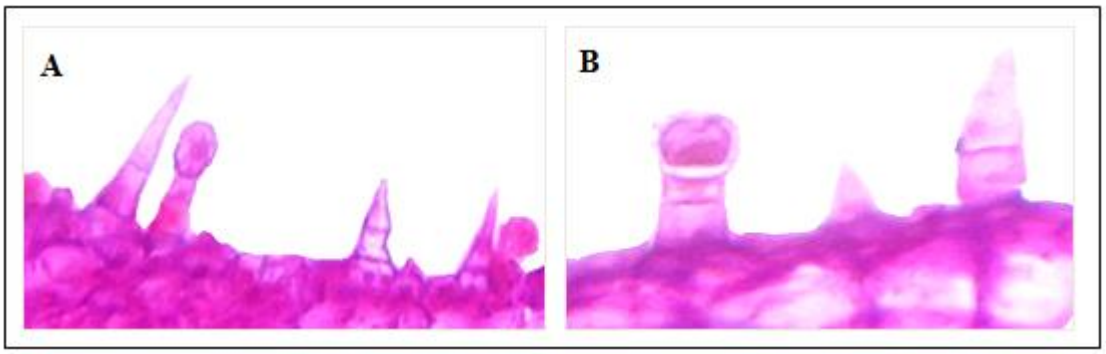

Fig.5. Types of trichomes accompanied apical internode of the main stem of *Salvia splendens*.
A and B: Non-glandular uniserate trichomes of one or two cells and glandular capitate trichomes with a head of one cell. (X 640)

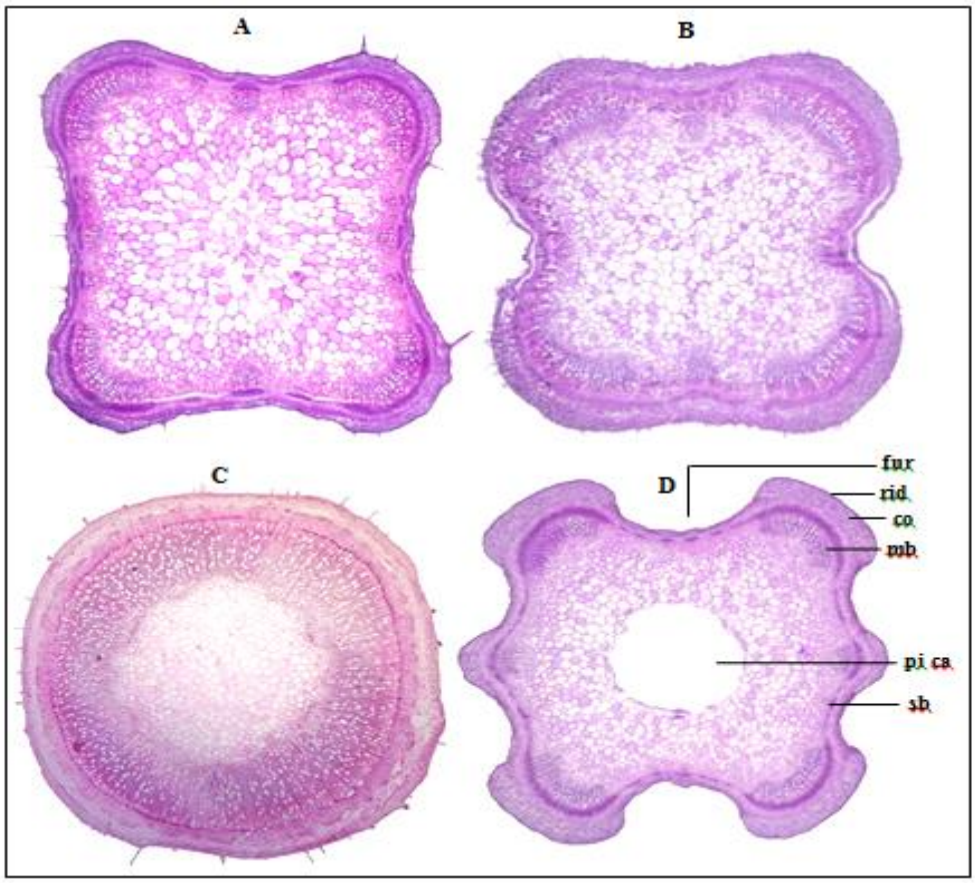

Fig. 6. Transverse sections through the median internode of the main stem of the four studied plant species of Salvia at the age of five months. (X 22).
S. coccinea B. S. farinacea C. S.officinalis D. S.splendens. Details: co, cortex; fur, furrow; mb, main bundle; pi ca, pith cavity; rid, ridge and sb, small lateral bundle.

The collateral bundles of *S. coccinea, S.farinacea* and *S.splendens* are arranged in a ring, being separated from one anther by ground tissue. The bundles varied in size; the bundles at the ridges are larger than the others. The cambial ring is observed between phloem and xylem. In case of *S.officinalis*, secondary growth takes place in a continuous cylindrical form. The secondary xylem has an increased amount of vessels present in nearly radial rows and the ground tissue where the vessels are embedded is formed of lignified parenchyma cells intermingled with small groups of fibers. The primary xylem is recognized abutting the pith.

ISSN: 0975-8585

The pith consists of polygonal parenchyma cells which tend to decrease in size towards the periphery and enlarge in size at the center. Stem of *S.splendens* becoming hollow; *i.e.,* a cavity is present in the central region and represents about 35% of the whole section.

The above mentioned description of stem of genus *Salvia* agrees with that stated by Metcalfe and Chalk (1957), Bagherpour *et al.* (2010) and Salimpour *et al.* (2012).

The leaf

The leaf blade

Leaf blade structure using light microscopy

The anatomical structure of leaf blades which represent simple leaves develope on the main stem of the four studied plant species of *Salvia* was investigated in form of transverse sections (Figs.7and 8). It is clear that, the leaf blade of *Salvia* species, 5 months old, consists of two epidermal layers (adaxial and abaxial) with a mesophyll in between. Both epidermal layers are uniseriate, composed of compactly arranged rectangular or barrel cells and covered with a thin cuticle layer. Trichomes and stomata are present on both surfaces. The mesophyll is differentiated into palisade and spongy cells. The palisade tissue is present towards the upper epidermis and consists of 2 layers of cells which elongated perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies about one-half of the whole thickeness of the mesophyll. The spongy tissue occurs towards the lower epidermis and composed of 3-4 layers of chlorenchymatous loosely arranged cells with obvious intercellular spaces.

It is worthy to note that, the leaf blade on both sides of the midrib of *S.officinalis* is corrugated in shape; having distinct ridges and furrows.

At the midrib region, the upper epidermis is almost flat in *S.coccinea, S.farinacea* and *S.officinalis* while the lower one is convex. In case of *S.splendens*, both epidermis are convex. The epidermal cells are oval in shape and followed by 1-2 layers of collenchyma cells.

The vascular bundle of the principle vein is relatively large in size and accompanied from above and below by parenchyma cells of the ground tissue and the vascular bundle is oriented with the xylem directed toward the adaxial surface and the phloem toward the abaxial one. The xylem consists of about 12-20 parallel rows according to the species, each xylem row comprise 5-7 vessels.

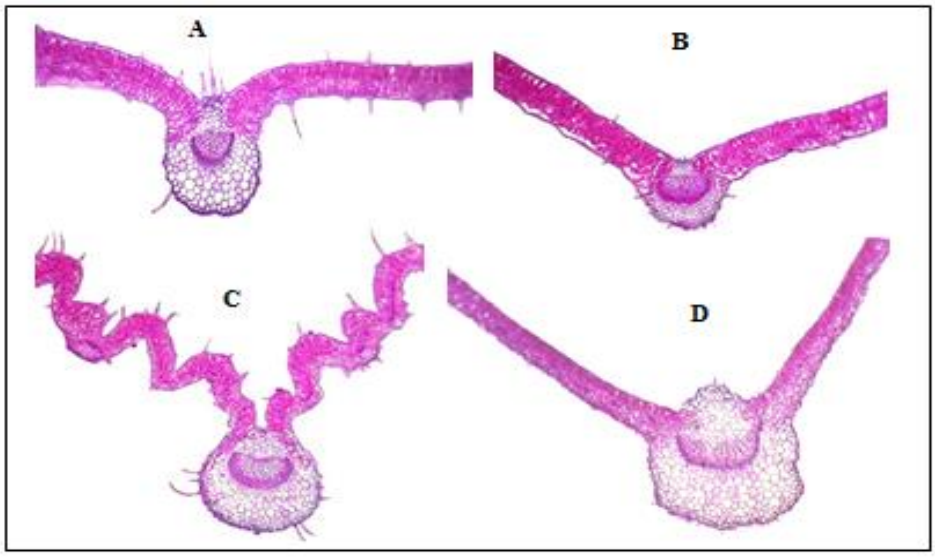

Fig. 7.Transverse sections through the blade of leaf develops at main stem median portion of the four studied plant species of Salvia aged five months. (X 40).
S. coccinea B. S. farinacea C. S.officinalis and D. S.splendens.

ISSN: 0975-8585

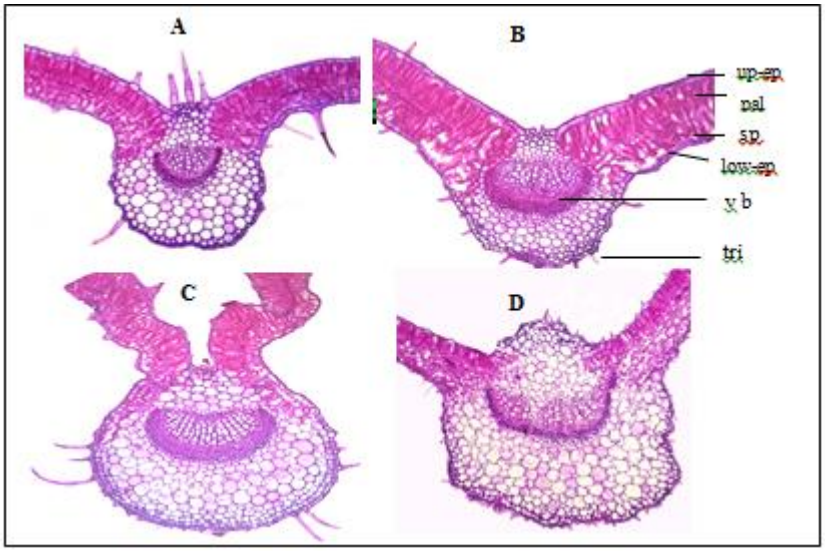

Fig.8.Transverse sections showing magnified portions through the blade of leaf develops at main stem median portion of the four studied plant species of Salvia aged five months. (X 100).
A. *S. coccinea* B. *S. farinacea* C. *S.officinalis* and D. *S.splendens*. Details: low-ep, lower epidermis; pal,palisade tissue; sp, spongy tissue; tri, trichome; up-ep, upper epidermis and v b, vascular bundle.

Leaf epidermal structure using scanning electron microscopy

Leaves of the four studied species of *Salvia* were subjected to scan electron microscopy to bring to light more information about their surface features. It is obvious that, there are two types of glandular trichomes; peltate and capitate type which are different in head structure as well as non-glandular ones. The peltate trichomes of studied plant species of *Salvia* compose of one base cell, one stalk cell (short stalk) and a large secretory head with four cells. This type is observed in *S. coccinea*, *S. farinacea* and *S.splendens*.

The capitate trichomes have similar base and stalk cells but a head of one broad cell (unicellular head) which are present in *S. farinacea*, *S.officinalis* and *S.splendens* (Figs. 9 to 12 and 15)**.** Non-glandular trichomes of *S. coccinea, S. farinacea* and *S.officinalis* are uniseriate, pointed, straight or curved, consist of 1-4 cells. In some trichomes, the second cell is shriveled. Moreover, the surface of non-glandular trichomes of the three studied plant species of *Salvia* is adorned with micropapillae (Figs. 9, 11 and 12). While the non-glandular trichomes of *S.splendens* are uniseriate unicellular; compose of one cell (Fig. 15).

It is realized from Fig (13) that both leaf surfaces of *S.officinalis* show a high distribution of non-glandular trichomes, these trichomes appeare in the form of groups on the upper surface but the lower surface being crowded and overlapping.

Scanning electron micrographs of both leaf surfaces of the four studied plant species of *Salvia* (Figs. 16 to 19) show that the epidermal cells have irregular shape with wavy margine. Stomata occur on both surfaces, being more numerous on the lower epidermis than on the upper one. In *S. coccinea, S. farinacea* and *S.officinalis*, stomata of anomocytic type (without any subsidiary cells) develop on both leaf surfaces. Worthy to note that, stomata of *S. farinacea* form at a depressed level compared with the remainder of epidermal cells. In case of *S.splendens* different types of stomata are found on the abaxial surface, they are anisocytic type (cruciferous) with three subsidiary cells (unequal in size) enclosing the guard cells and diacytic type (caryophyllaceous); *i.e.,* guard cells surrounded by two subsidiary cells oriented perpendicular to the pore of stoma. The above mentioned description of *Salvia* species epidermal cell features is in agreement with that given by Metcalfe and Chalk (1957) and Anon. (2012).

The leaf petiole

Microphotographs shown in Fig. (20) depict structure of the leaf petiole of the four studied *Salvia* species in transverse sections. It is clear that, the shape of petiole varies in different species; being reniform-

ISSN: 0975-8585

shaped in *S.coccinea* and *S.splendens*, U-shaped in *S.farinacea* and boat- shaped in *S.officinalis.* The adaxial surface of petiole is almost flat to concave with two wings at its corners while the abaxial surface is convex.

The petiole is bounded by a uniseriate epidermis of nearly barrel to rectangular shaped cells. The outer walls of the epidermis are somewhat thickened and covered with a thin layer of cuticle. Stomata and trichomes of various kinds are present. There are 2-3 layers of collenchyma cells underlying the epidermis. The ground tissue consists mostly of relatively large polygonal parenchyma cells with small triangular intercellular spaces.

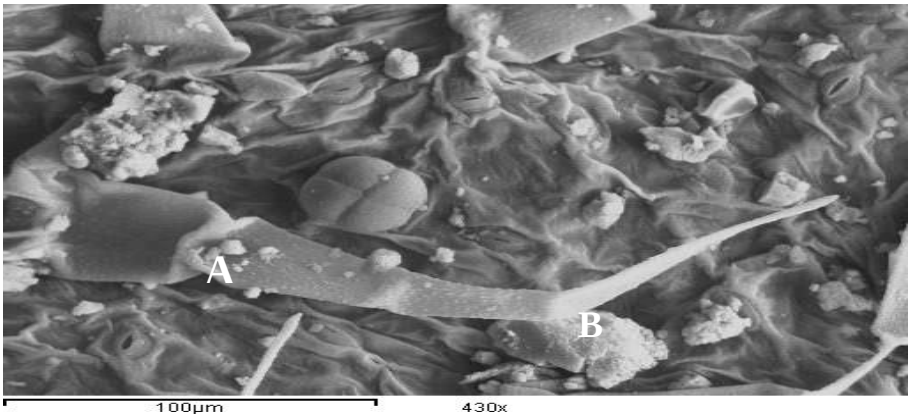

Fig. 9. A scanning electron micrograph of the adaxial surface of *Salvia coccinea* leaf blade showing,
A. Glandular trichome of a peltate type with four cells in the secretory head.
B. Uniseriate, multicellular and unbranched non-glandular trichome. (Scale bar = 100 µm)

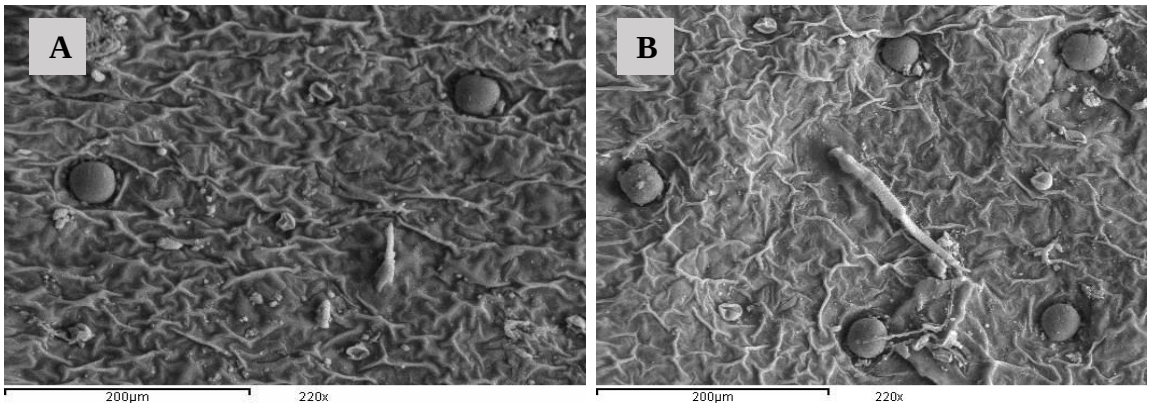

Fig. 10. Scanning electron micrographs of leaf blade of *Salvia farinacea* showingglandular trichomes develop on both of the leaf epidermal surfaces, being more abundant on the abaxial one.
A. Adaxil surface B. Abaxial surface (Scale bar = 200 µm).

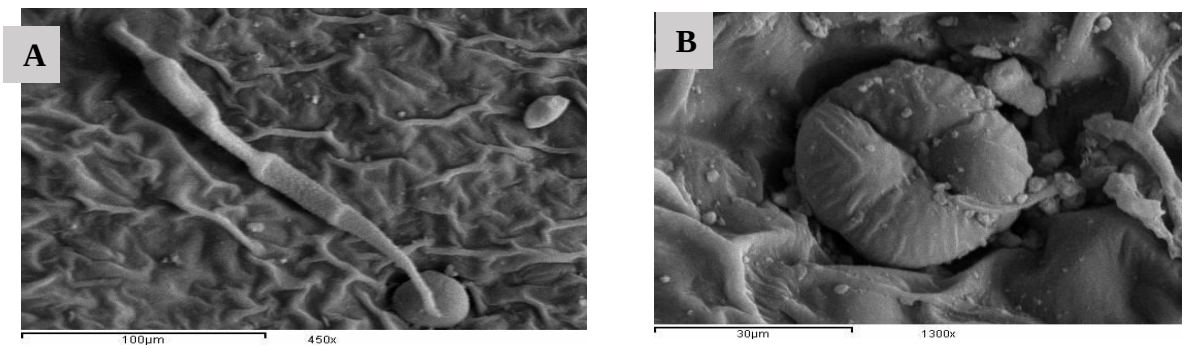

Fig. 11. Scanning electron micrographs of the abaxial surface of *Salvia farinacea* leaf blade showing ,
A. Uniseriate, multicellular and unbranched non glandular trichome. (the second cell shriveled). (Scale bar = 100 µm). B. Glandular trichome of a peltate type with four cells in the secretory head. (Scale bar = 30 µm).

ISSN: 0975-8585

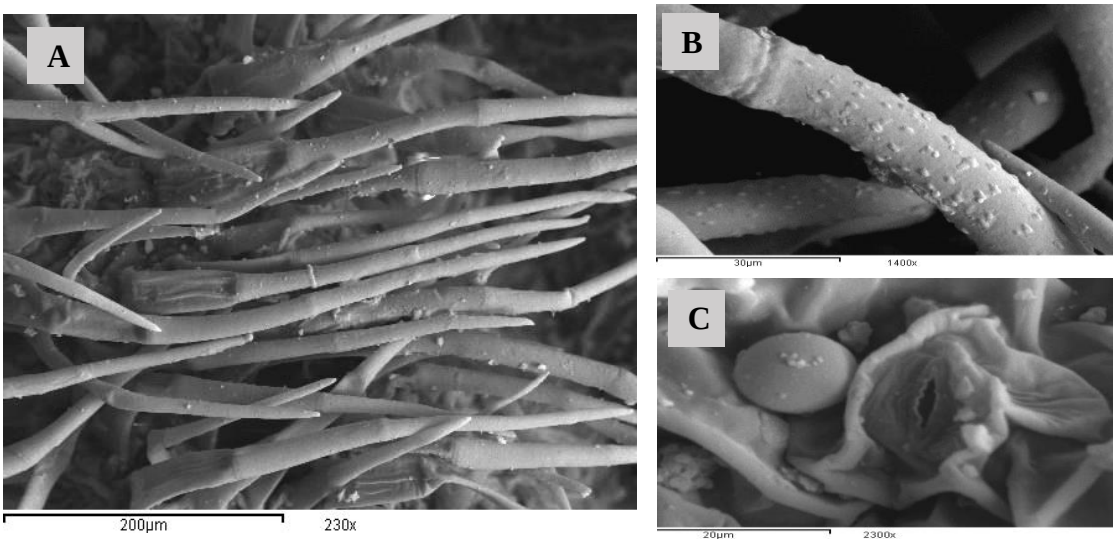

Fig. 12. Scanning electron micrographs of the adaxial surface of *Salvia officinalis* leaf blade showing ,
A. Uniseriate, multicellular and unbranched non glandular trichome. (Scale bar = 200 µm).
B. Magnified portion of A (Scale bar = 30 µm).
C. Glandular trichome of a capitate type. (Scale bar = 20 µm).

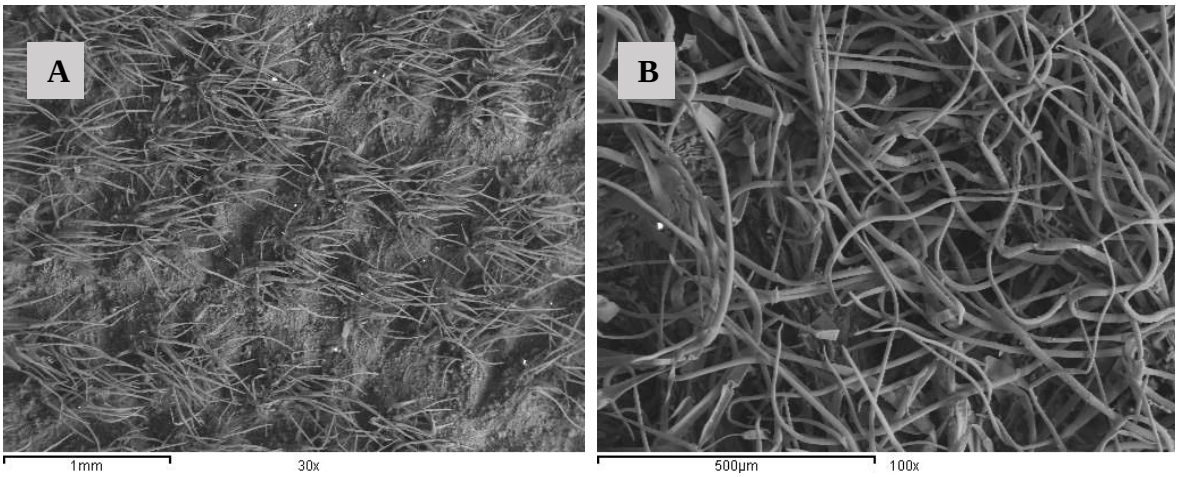

Fig. 13. Scanning electron micrographs of leaf blade of *Salvia officinalis* showing high distribution of non-glandular trichomes .
A. Adaxial surface (Scale bar = 1mm).
B. Abaxial surface (Scale bar = 500µm).

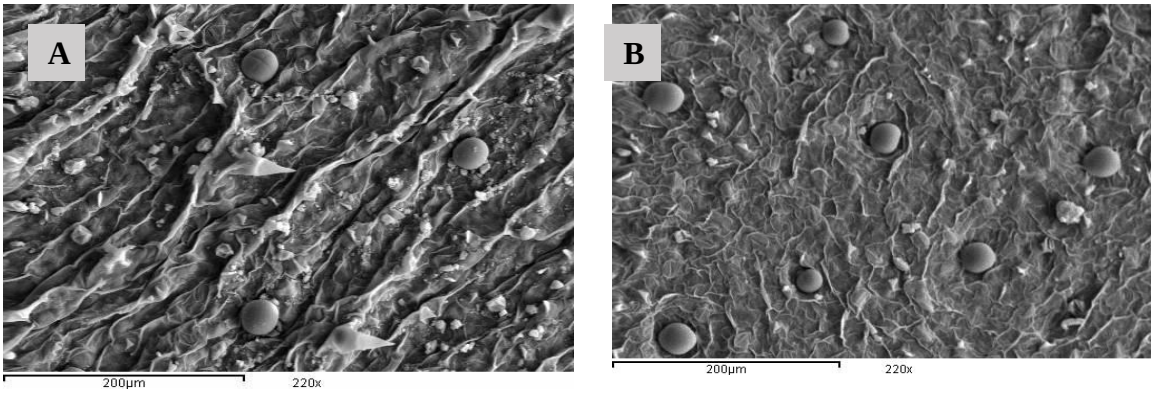

Fig. 14. Scanning electron micrographs on both of leaf blade surfaces of *Salvia splendens* showing glandular trichome develop on both of the leaf epidermal surfaces, being more abundant on the abaxial one. A. Adaxil surface B. Abaxial surface (Scale bar = 200 µm).

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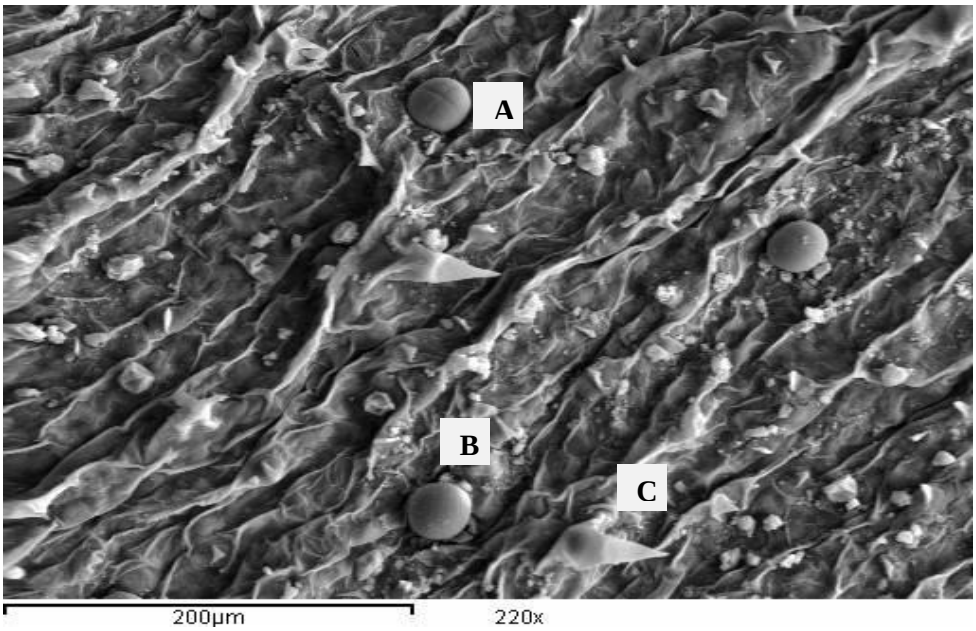

Fig. 15. Scanning electron micrograph of the adaxial surface of *Salvia splendens* showing;
A. Glandular peltate trichome with a head of four cells.
B. Glandular capitate trichome with a head of one cell.
C. Uniseriate, unicellular non glandular trichome. (Scale bar = 200 µm).

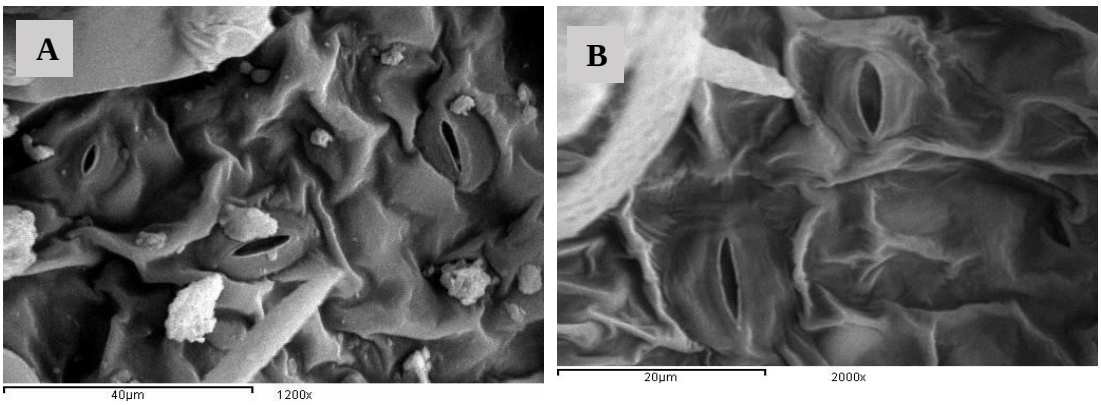

Fig. 16. Scanning electron micrographs of leaf blade of *Salvia coccinea* showing stomata of anomocytic type.
A. Adaxial surface (Scale bar = 40 µm). B. Abaxial surface (Scale bar = 20 µm).

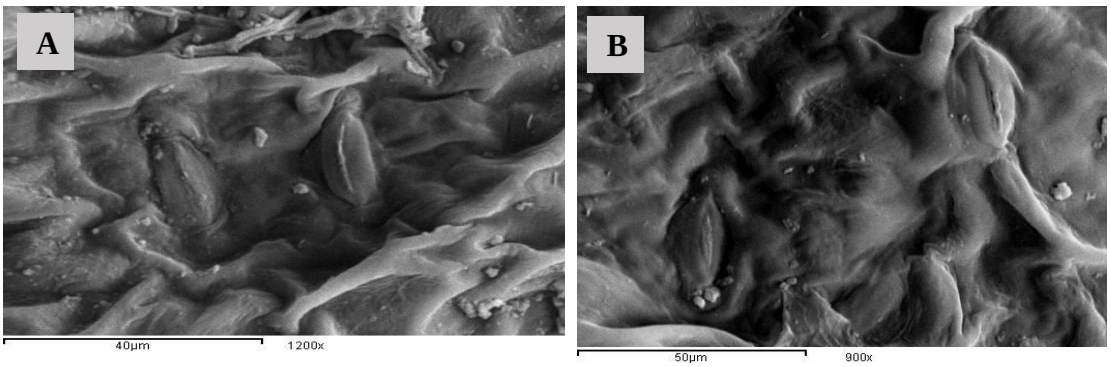

Fig. 17. Scanning electron micrographs of leaf blade of *Salvia farinacea* showing stomata of anomocytic type formed at a depressed level compared with other species.
A. Adaxial surface (Scale bar = 40 µm). B. Abaxial surface (Scale bar = 50µm)

ISSN: 0975-8585

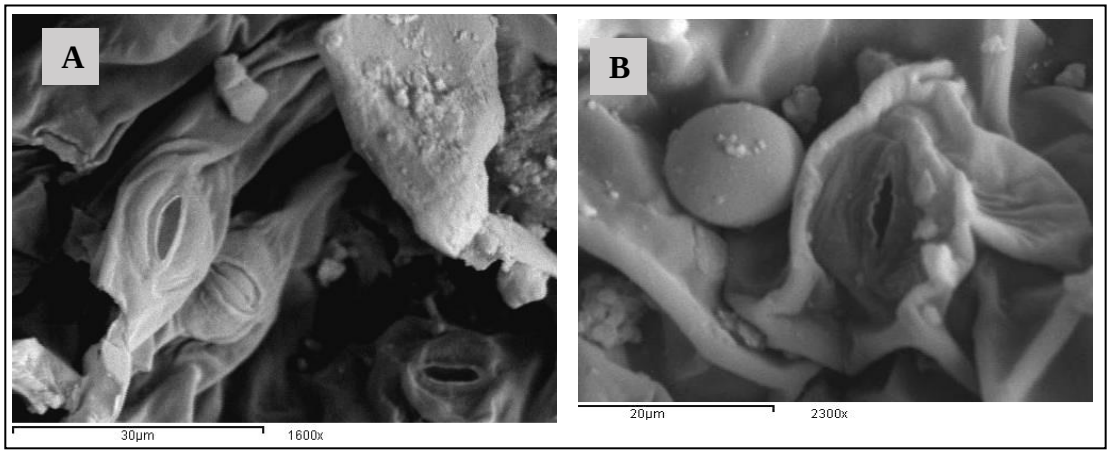

Fig. 18. Scanning electron micrographs of the adaxial surface of *Salvia officinalis* leaf blade showing; A. Stomata of anomocytic type. (Scale bar = 30 µm). B. Glandular trichome of a capitate type. (Scale bar = 20 µm).

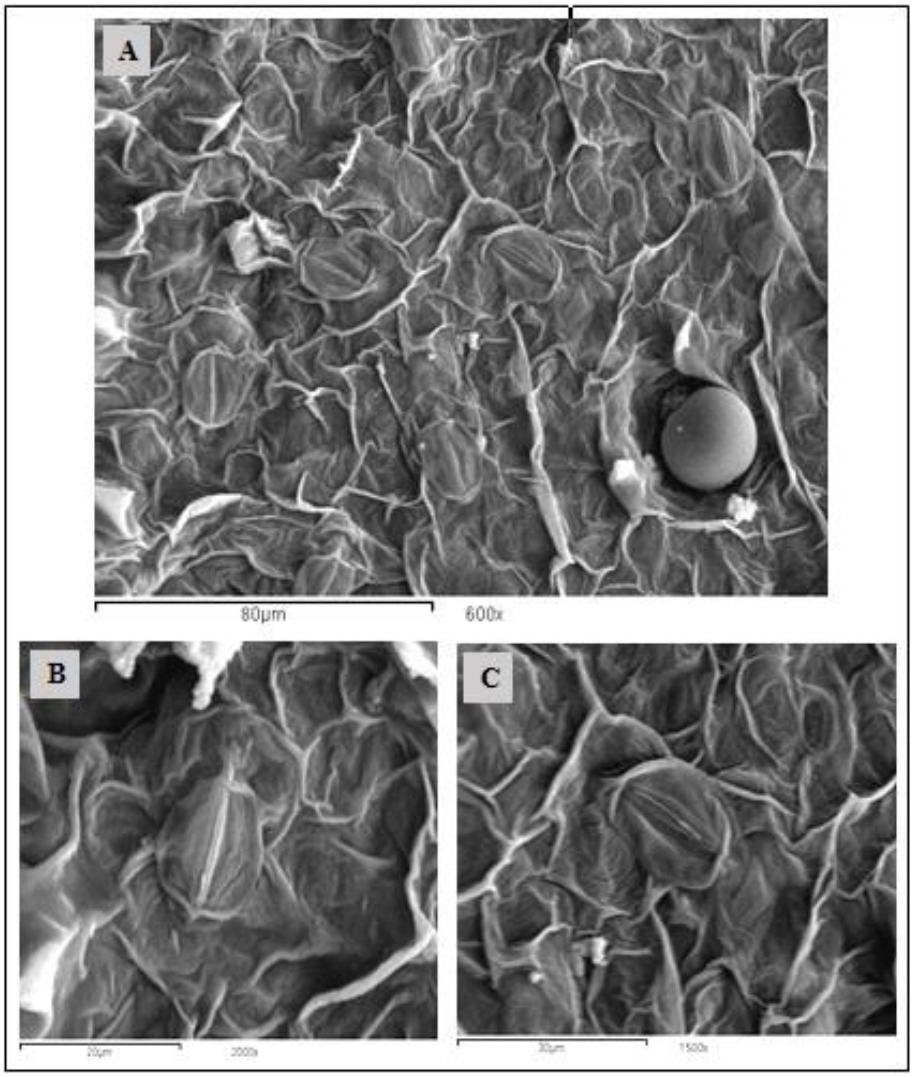

Fig. 19. Scanning electron micrographs of the abaxial surface of *Salvia splendens* showing, A. different types of stomata (Scale bar = 80 µm). B. Stoma of anisocytic type (Scale bar = 20 µm). C. Stoma of diacytic type (Scale bar = 30 µm).

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It has been observed that, the number of vascular bundles of petiole varies depending upon species. In *S.coccinea*, there is a large single vascular bundle located in the center of the section and one small subsidiary vascular bundle in each wing. In *S. farinacea*, there are two vascular bundles located in the center and three small subsidiary vascular bundles in each petiolar wing. In *S.officinalis,* there are four vascular bundles embedded in the ground tissue. The largest one is located in the center of section and three small traces are present at its sides, beside small accessory bundle in each wing. In *S.splendens*, there are three main bundles embedded in the ground tissue and one subsidiary vascular bundle at each wing.

The main vascular bundle of petiole of all studied *Salvia* species is arc in shape and oriented with the xylem directed towards the adaxial surface and the phloem towards the abaxial one. Each bundle is surrounded by a sheath of one layer of parenchymatous cells.

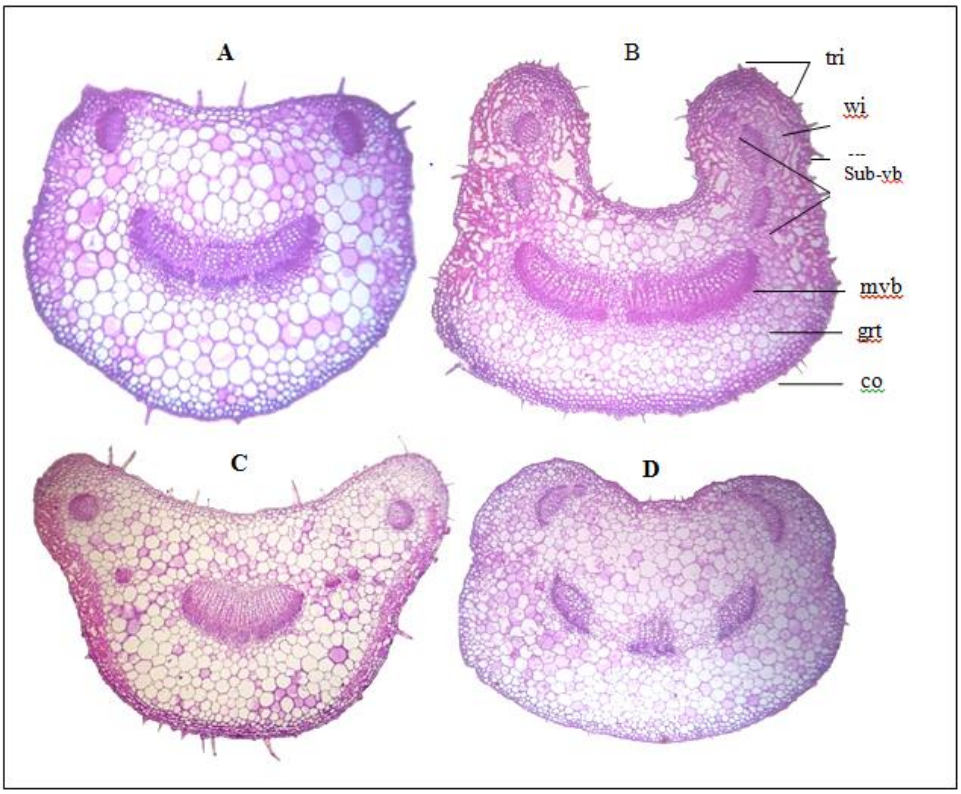

Fig.20. Transverse sections through the leaf petiole of the four studied plant species of *Salvia* at the age of five months. (X 48)

A. *S. coccinea* B. *S. farinacea* C. *S.officinalis* and D. *S.splendens*

Details: co, collenchymas; ep, epidermis; grt, ground tissue; mvb, main vascular bundle; sub-vb, subsidiary vascular bundle; tri, trichome and wi, wing.

C. Structure of the floral bud

Transverse sections through the floral bud of the four studied species of *Salvia* are shown in Figure (21). It is clear that the flower is perfect, hermaphrodite and zygomorphic. The calyx consists of five united sepals comprised two epidermal layers with ground tissue in between and there are numerous small vascular bundles extending through the ground tissue. It is obvious that the calyx is covered with trichomes of different types.

The corolla tube composed of two epidermal layers surrounding 2-3 layers of loosely parenchymatous cells forming the mesophyll. Many traces are extending through the mesophyll.

The androecium comprise two stamens, each consists of two lobed anther which separated by elongated connective. The topmost anther lobe is fertile whereas the lower lobe is sterile.

ISSN: 0975-8585

Gynoecium as seen in Figure (22) composed of two united carpels, four chambered and gynobasic style. There is a single ovule in each loculus with basal placentation.

The nutlet

Nutlet structure using light microscopy

Transverse sections of nutlets of studied *Salvia* species are showen in Figure (23). It is obvious that the nutlets are ovate to trigonous in outline and comprised one seed with dicotyledonous embryo.

The nutlet pericarp of all studied *Salvia* species differentiated into three main regions; the exocarp (outer epidermis), mesocarp and endocarp (inner epidermis) followed by seed testa which envelopes small amount of endosperm and the embryo.

The exocarp consists of a single layer of columnar sclerenchymatic cells. Below the exocarp, there is the mesocarp region which composed of several layers of paranchymatous cells and it can be easily distinguished in *S. coccinea* (7 layers) and *S.splendens* (14 layers) compared to *S. farinacea* and *S.officinalis* (2-3 layers). The endocarp consists of a single layer of tubular or pillar-like cells. This agrees with the pericarp structure of the genus *Salvia* given by Oran (1997) studied the nutlet anatomy of the genus *Salvia* L. and reported that the pericarp of the nutlet is made of three distinct regions namely; epicarp, mesocarp and endocarp. The epicarp consists of the epidermis and the hypodermis layers. The mesocarp consists of undifferentiated parenchymatous tissue. The endocarp consists of sclerenchymatic tissue, either columnar or with osteosclerides. The testa underlying the pericarp consists of a thin layer of which envelops the endosperm and the embryo.

Nutlet characters by using scanning electron microscopy

Nutlets of the four studied *Salvia* species as seen by scanning electron microscope (Figure 24) are different in shape; elongated ovoidal or pyriform (pear-like) in *S.coccinea*, ovoid in *S.farinacea*, spherical to ovoid in *S.officinalis* and elliptical with uneven apex in *S.splendens*.

The epidermal cells of the nutlets are irregular and papillate (Figure, 25). The anticlinal walls are deeply or slightly depressed and usually 5-6 gonal. The outer periclinal cell walls are flat with rounded projections (papillae). The hilum is ovate in *S.coccinea* and *S.farinacea*, nearly rounded in *S.officinalis* and irregular- shaped in *S.splendens* (Figure, 26).

The above description of the nutlet shape is more or less in agreement with that given by Marin *et al.* (1996).

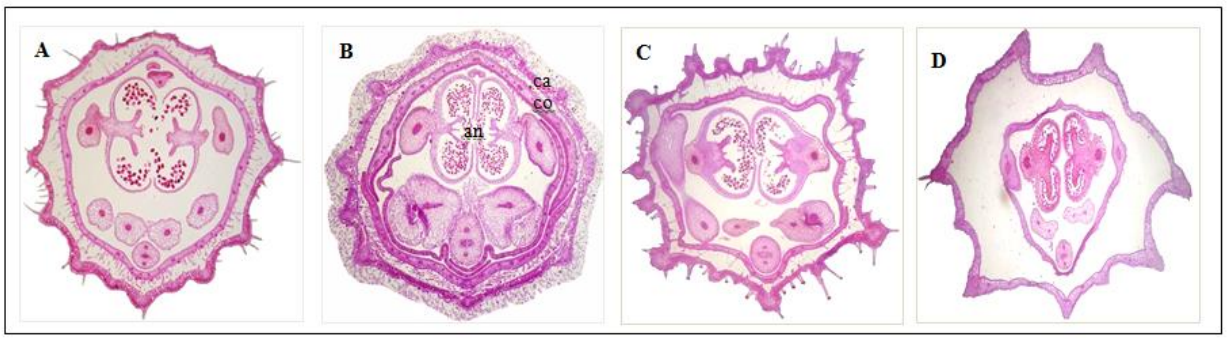

Fig.21. Transverse sections through the apical portion of the floral buds showing stamens of the four plant species of *Salvia*. (X 40)
A. *S. coccinea* B. *S. farinacea* C. *S.officinalis* and D. *S.splendens* / Details: an, anther; ca, calyx and co, corolla.

ISSN: 0975-8585

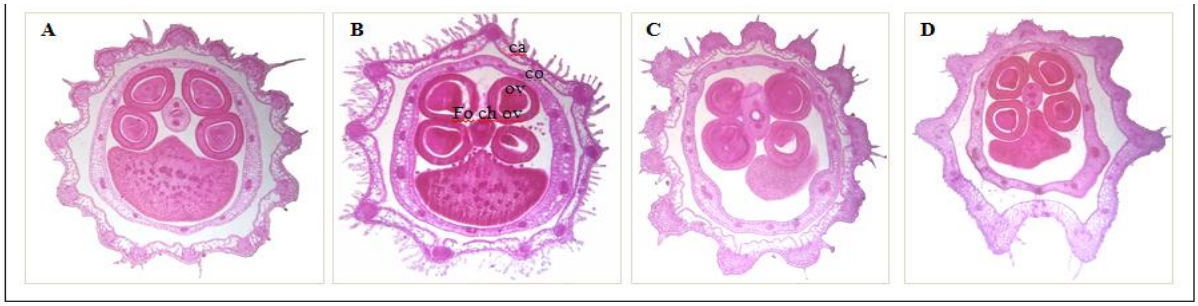

Fig.22. Transverse sections through the basal portion of the floral bud showing ovary of the four studied plant species of *Salvia*. (X 60)
***S. coccinea* B. *S. farinacea* C. *S.officinalis* and D. *S.splendens* Details: ca, calyx; co, corolla; fo ch ov, four chambered ovary and ov, ovule.**

Fig.23. Transverse sections through nutlet of the four studied plant species of *Salvia,* at fruiting stage . A. *S. coccinea* , B. *S. farinacea* (X 65) C. *S.officinalis* D. *S.splendens* (X 60). Details: cot, cotyledons; endo, endocarp; exo, exocarp and meso, mesocarp.

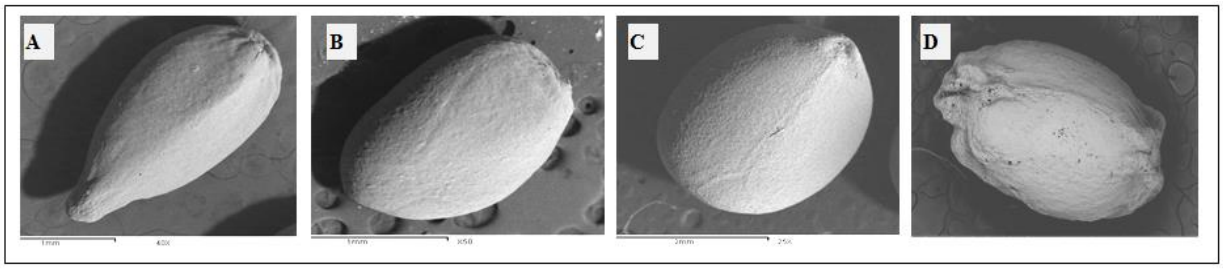

Fig.24. SEM micrographs of the four studied plant species of *Salvia,* showing nutlet (seed) shape. A. *S. coccinea* (Scale bar = 1mm) : Pyriform,
A. *S. farinacea* (Scale bar = 1mm) : Ovoid, C. *S.officinalis* (Scale bar = 2mm) : Spherical to ovoid and D. *S.splendens* (Scale bar = 1mm) : Elliptical with uneven apex.

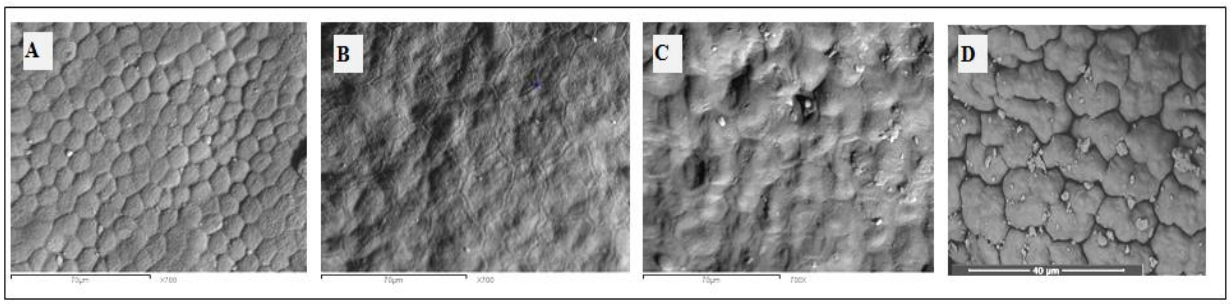

Fig.25. SEM micrographs of nutlet of the four studied plant species of *Salvia,* showing nutlet sculpture patterns.
A. *S. coccinea* (Scale bar = 70 µm) B. *S. farinacea* (Scale bar = 70 µm)
B. *S.officinalis* (Scale bar = 70 µm) D. *S.splendens* (Scale bar = 40 µm)

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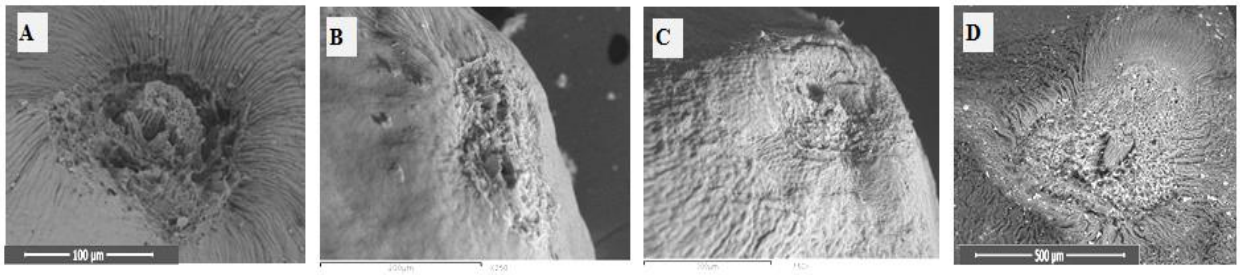

Fig.26. SEM micrographs of nutlet of the four studied plant species of *Salvia,* showing nutlet hilum region.
A. *S. coccinea* (Scale bar = 100 µm) B. *S. farinacea* (Scale bar = 200 µm)
***C. S.officinalis* (Scale bar = 300 µm) D. *S.splendens* (Scale bar = 500 µm)**

Molecular characterization

Molecular fingerprint of the four studied plant species of *Salvia* was made for the sake of proper identifying and defining limits of relationship among them. This was carried out by electrophoretic separation of seed storage proteins using SDS-PAGE.

The results of SDS-PAGE of total protein in seeds of the four studied *Salvia* species are given in Figure (27) and Table (1). From the SDS-PAGE analysis of 11 bands were detected with different molecular weights (MW).

Regarding these 11 bands, *S. coccinea*, *S. farinacea, S.officinalis* and *S. splendens* recorded 6, 5, 10 and 6 bands; respectively. *S.officinalis* recorded four monomorphic (unique) bands at molecular weights 177, 98, 75 and 52 KD and *S.splendens* recorded only one monomorphic band at molecular weight 25 KD, while *S.coccinea* and *S.farinacea* did not record any monomorphic band. It is clear that, 4 common bands were present in all studied species at molecular weights 70, 56, 43 and 29 KD. *S. coccinea* shared both *S.farinacea* and *S.splendens* in 5 bands, in the meantime *S. coccinea* and *S.officinalis* shared 6 bands. However, *S. farinacea* and *S. officinalis* shared 5 bands, while *S.farinacea* and *S. splendens* shared 4 bands. Whereas, *S.officinalis* and *S.splendens* shared 5 bands. Similarity index of total protein in seeds of the four studied *Salvia* species based on SDS-PAGE is shown in Table (2). The dendrogram representing the electrophoretic banding patterns of total protein in seeds of the studied species is given in Figure (28).

The dendrogram indicates that the four tested plant species can be classified into 3 main clusters. The first cluster contained *S.officinalis*, the second cluster included *S.splendens* while the third cluster grouped both *S.coccinea* and *S.farinacea* together. Such results proved that the latter two species are highly similar (83%) compared to other studied species. Followed that, the relationship between *S.splendens* and each of *S.coccinea* (71%) or *S.farinacea* (57%). However, the relationship was lesser between *S.officinalis* and any of the other three studied species.

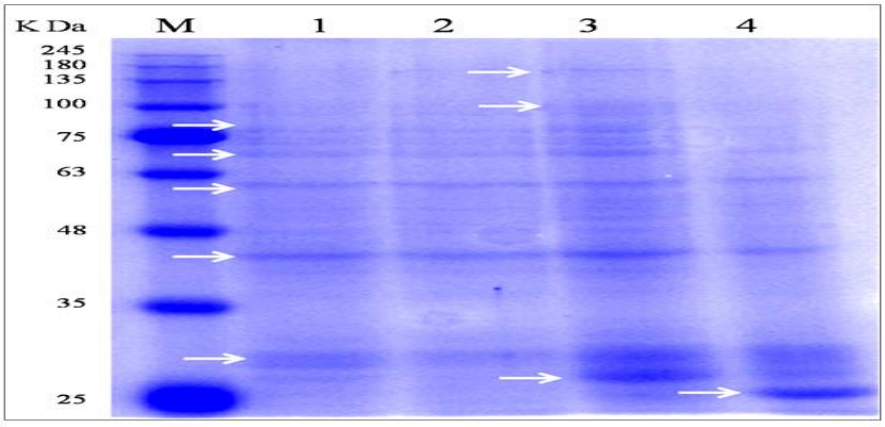

Fig. 27. SDS-PAGE of total proteins in seeds of the four studied plant species of *Salvia*.
(M) protein marker, (1) *S.coccinea*, (2) *S. farinacea,* (3) *S.officinalis* and (4) *S.splendens.*

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Table 1. Densitometer analysis of total protein SDS-PAGE in seeds of the four studied plant species of Salvia showing band number and molecular weight M.W. (KD).

Band No.	M.W. (KD)	Recorded protein bands			
		S. coccinea	*S. farinacea*	*S.officinalis*	*S.splendens*
1	177	0	0	1	0
2	98	0	0	1	0
3	78	1	1	1	0
4	75	0	0	1	0
5	70	1	1	1	1
6	56	1	1	1	1
7	52	0	0	1	0
8	43	1	1	1	1
9	29	1	1	1	1
10	27	1	0	1	1
11	25	0	0	0	1

1 = Present and 0 = Absent.

Table 2. Similarity matrix computed with Jaccard coefficient of the four studied plant species of Salvia based on SDS- PAGE.

	S. coccinea	*S. farinacea*	*S.officinalis*	*S.splendens*
S. coccinea	**1.0**	**0.83**	**0.60**	**0.71**
S. farinacea		**1.0**	**0.50**	**0.57**
S.officinalis			**1.0**	**0.46**
S.splendens				**1.0**

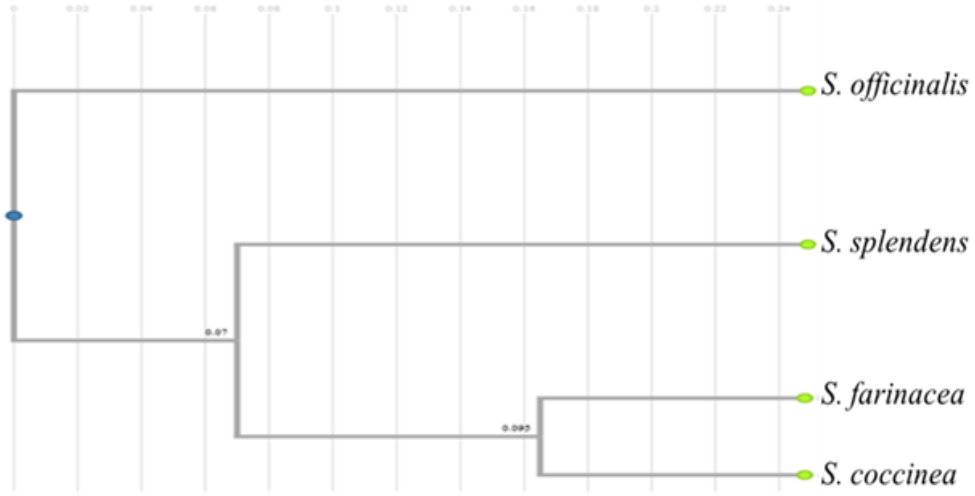

Fig.28. Dendrogram of similarity obtained by the UPGMA method showing relationship among the four studied plant species of Salvia based on SDS- PAGE.

Diagnostic characters of studied species

Data in Table (3) summarize diagnostic characters of the four studied species of *Salvia* including anatomy and molecular characteristics. It is clear that, many characters seem to be common in all of them; *i.e,* diagnostic at the genus level and other characters were unique for each species. That was made for sake of proper delimitation and differentiation among species.

ISSN: 0975-8585

Table 3. Diagnostic characters of the four studied plant species of *Salvia*.

Characters	*S. coccinea*	*S. farinacea*	*S.officinalis*	*S.splendens*
Histology Main stem:				
Apical internode:				
Stem outline	Tetragonal	Tetragonal	Tetragonal	Hexagonal
Number of bundles at the ridges	4	4	4	6
Trichomes: Non-glandular	Uniseriate (uni and multicellular)	Uniseriate (bicellular and multicellular)	Uniseriate (bicellular and multicellular)	Uniseriate (uni and bicellular)
Glandular	Capitate type (head of 1cell)	Capitate (uni and bicellular) Peltate (head of 4 cells)	Peltate type (head of 4 or more cells)	Capitate type (head of 1cell)
Median internode:				
Stem outline	Quadrangular	Quadrangular	Cylindrical	Hexangular
Secondary xylem	Absent	Absent	Present	Absent
Pith cavity	Absent	Absent	Absent	Present
Leaf : Trichomes				
Non-glandular	Uniseriate, multicellular	Uniseriate, multicellular	Uniseriate, multicellular	Uniseriate, unicellular
Glandular	Peltate type	Peltate and capitate types	Capitate type	Peltate and capitate types
Stomata	Anomocytic	Anomocytic	Anomocytic	Anisocytic and diacytic
Petiole: Shape	Reniform	U-shaped	Boat-shaped	Reniform
Total number of vascular bundles	3 bundles (one in the center and one at each wing)	8 bundles (two in the center and three at each wing)	6 bundles (four in the center and one at each wing)	5 bundles (three in the center and one at each wing)

Table 3: cont.

Nutlet:				
Nutlet outline	Trigonous	Ovate	Trigonous	Trigonous
Parenchyma layers / msocarp	7 layers	2-3 layers	2-3 layers	14 layers
Hilum shape	Ovate	Ovate	Nearly rounded	Irregular- shaped
Molecular characteristics:				
Protein electrophoresis using (SDS-PAG):				
No. of protein bands	6	5	10	6
Shared number of protein bands	6	5	6	5
Monomorphic bands of protein	0	0	4	1

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REFERENCES

[1] Alberto, C.M.; Sanso, A.M. and Xifreda, C.C. (2003). Chromosomal studies in species of *Salvia* (Lamiaceae) from Argentina. Bot. J. Linn. Soc., 141: 483-490.

[2] Anonymous (2012). *Salvia officinalis* for homoeopathic preparations. The General

[3] Chapters and General Monographs of the European Pharmacopoeia (French

[4] Pharmacopoeia), p. 1-5.

[5] Bagherpour, S.; Kahraman, A.; Doğan, M.; Celep, F.; Başer, B. and Pehlivan, S. (2010). The anatomical and micromorphological characteristics of *Salvia vermifolia* (Section Aethiopis Bentham, Lamiaceae) from Central Anatolia, Turkey. Central European Journal of Biology. 5(6), 872-879.

[6] Cronquist, A. (1981). An Integrated System of Classification of Flowering Plants. Columbia University Press, N.Y., U.S.A., pp.915-924.

[7] Dahiya, B.S. (1979). Systematic Botany (Taxonomy of Angiosperms). Kalyani Publishers, New Delhi-Ludhiana.p.434.

[8] El-Sahhar, K.F.; Nassar, R.M. and Farag, H.M. (2016). Comparative botanical studies of some *Salvia* species (Lamiaceae) grown in Egypt. I- Morphological characteristics. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(3): 1985- 2000.

[9] Harley, R.; Atkins, S.; Budantzev, A.L.; Cantino, P.D.; Conn, B.J.; Grayer, R.; Harley, M.M.; Kok, R. De; Krestovskaja, T.; Morales, R.; Paton, A.J. and Upson, T. (2004). The Family and Genera of Vascular Plants. Vol. 7 Berlin : p. 167-275.

[10] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nuture, 227: 680-685.

[11] Marin, P.D.; Duletic, S. and Petkovic, B. (1996). Nutlet ornamentation in selected *Salvia* L. species (Lamiaceae). Flora Mediterranea. 6: 203-211.

[12] Masoud, S.; Alijanpoo, B. and Khayyami, M.(2010). Contribution to cytology of genus *Salvia* L. (Lamiaceae) in Iran. Vol. 63: 405-410.

[13] Metcalfe, C.R. and Chalk, L. (1957). Anatomy of The Dicotyledones, (vol.П). The Clarendon Press, Oxford. pp. 1041-1053.

[14] Nassar, M.A. and El-Sahhar, K. F. (1998). Botanical Preparations and Microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, 219 pp. (In Arabic).

[15] Oran, S. A. (1997). Nutlet anatomy of the genus *Salvia* L. in Jordan. Flora Mediterranean 7, 27-40.

[16] Özdemir, C. and Altan, Y. (2005). Morphological and anatomical characteristics of endemic *Salvia huberi* Hedge in Turkey. Bangladesh J. Bot. 34 (2): 95-100.

[17] Salimpour, F.; Ebrahimiyan, M.; Sharifnia, F. and Tajadod, G. (2012). Numerical taxonomy of eight *Salvia* L. species using anatomical properties. Annals of Biological Research, 3 (2):795-805.

[18] Simpson, M.G., (2010). Plant Systematics. Elsevier Academic Press, Second Edition Canada, p. 399-406.

[19] Studier, F.W. (1973). Analysis of bacteriophage T1 early RNAs and proteins of slab gels. J. Mol.Biol., 79: 237-248.